REVIEW ARTICLE Egypt. J. Med. Hum. Genet. Vol. 11, No. 1, May, 2010

Severe congenital neutropenia (Kostmann Syndrome)

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ABSTRACT

Severe congenital neutropenia (SCN), Kostmann syndrome is a heterogenous disorder of myelopoiesis characterized by severe chronic neutropenia, absolute neutrophil count (ANC) persistently below 0.50 x 109/L, with maturation arrest of neutrophil precursors in the bone marrow; and associated with serious recurrent bacterial infections from early infancy. Sepsis mortality is reduced by an advent of granulocyte colony stimulating factor (G-CSF) therapy.

More than 90% of patients respond to G-CSF therapy. However, hematopoietic stem cell transplantation has shown promise in the treatment of non-responders. About 60-80% of SCN cases are associated with constitutive mutations in one copy of the gene encoding neutrophil elastase ELA2. Myelodysplastic syndrome and acute myeloid leukemia (MDS/AML) have been reported. The hazard of MDS/AML increases significantly overtime. Approximately 10% of patients with severe congenital neutropenia develop AML. This is not thought to be the direct result of G-CSF therapy but related to the underlying disease itself.

Key Words:

Myeloid leukemia, congenital neutropenia, kostmann's syndrome, myelodysplastic syndrome.

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INTRODUCTION

Neutrophils play a vital role in protecting the body against invasion by bacteria. Severe chronic neutropenia is a general term for conditions with absolute neutrophil count (ANC) of less than 0.50x 109/L, lasting for months or years. Chronic neutropenia is caused by a variety of hematological, immunological, metabolic and infectious diseases[1].

The category of SCN was first clearly described by Kostmann in 1956 in Swedish kindred. An alternative name for Kostmann syndrome is infantile genetic agranulocytosis[2]. Kostmann syndrome represents an autosomal recessive, heterogenous hematological disorders, characterized by extremely low circulating neutrophils and maturation ar-

rest at the promyelocyte-myelocyte in the bone marrow. Neutrophil counts in these patients are typically <0.2x 109/L, with the majority presenting with life-threatening infection during the first 6 months of life. SCN with similar clinical features occurs as an autosomal dominant disorder and many sporadic cases also have been reported[3].

Other conditions grouped with severe congenital neutropenia (CN) include Shwachman-Diamond syndrome (SDS); inborn errors of metabolism (e.g., glycogen-storage disease, type Ib)[4]; severe combined immunodeficiency (SCID) syndromes; hyper-IgM syndrome and a few other conditions[3,32] Patients with clinically mild disease, generally tend to have a less abnormal bone marrow. The estimated frequency of these conditions is approximately 1-2 cases per million population.

Pathophysiology:

Neutropenia refers to an ANC that is two or more standard deviations below the age related mean. Normal neutrophil levels differ according to age and race. In general, an ANC is calculated by multiplying the total leukocyte count per milliliter by the percentage of neutrophils and immature neutrophil form. In general, an ANC of 1000–1500/mm3 indicates mild neutropenia; 500-1000/mm3 indicates moderate neutropenia; and less than 500/mm3 indicates severe neutropenia. This classification is useful for predicting risk of infections.

Mortality/Morbidity:

The mortality rate is 70% within the first year of life in the absence of medical intervention with G-CSF and/or hematopoietic stem cell transplantation.

The classic Kostmann syndrome is recognized in early infancy with an equal incidence in males and females. Severe neutropenia is brought to clinical attention after an initial infection which typically occurs shortly after birth. Symptoms of Kostmann include the followings: Temperature instability in newborn period, fever, irritability and localized sites of infection.

Physical and signs of Kostmann syndrome include the followings: Oral ulcers, gingivitis, pharyngitis, sinusitis and/or otitis media, bronchitis and/or pneumonia, cellulitis, cutaneous abscess and/or boils, perianal abscess, lung or liver abscess, enteritis with chronic diarrhea and vomiting. Bacteremia and/or septicemia occur, most commonly from streptococci or staphylococci; other organisms include Pseudomonas, fungi and in rare cases Clostridium species.

Pathogenesis of congenital neutropenia:

The underlying genetic defect of SCN is still only partially understood. Some investigators have proposed that Kostmann syndrome represents a defect in the regulation or production of granulocyte colony-stimulating factor (G-CSF).

An abnormal G-CSF-induced intracellular signal transduction pathway has been suggested as a potential cause of the underlying genetic defect. Neutrophils from patients are shown to have dramatically increased levels of 2 cytosolic protein tyrosine phosphatases that contain Src-homology 2 domain (SH2

domain): Anti-Src Homology Phosphatase-1 (SHP-1) and Src Homology Phosphatase 2 (SHP2). One hypothesis is that over expression of these proteins, which are involved in cytokine receptor signaling, plays a role in altering intracellular signal transduction processes.

A selective decrease of B-Cell Lymphoma-2 (BCL-2) expression in myeloid cells and an increase in apoptosis in bone marrow progenitor cells have been observed. The primary function of BCL-2, a mitochondria-targeted protein, is the prevention of Cytochrome release. Cytochrome has the capability of activating a cytosolic caspases cascade. Caspases are integral proteolytic enzymes involved in cellular apoptosis. They are activated through one of two pathways:

1. An extrinsic, death receptor-dependent pathway or.
2. An intrinsic, mitochondria-dependent pathway. Mitochondrial release of Cytochrome c serves to initiate the cytosolic caspases cascade through the second pathway. The decreased BCL-2 results in an enhanced release of Cytochrome c, which then perpetuates the caspases cascade leading to more pronounced apoptosis.

In Kostmann syndrome, the G-CSF receptors are expressed on myeloid cells in slightly increased numbers and the binding affinity for G-CSF to its receptors is normal. This is in contrary to the original theory that the underlying Kostmann defect was related to either decreased G-CSF production or diminished binding of G-CSF to its receptor.[5]

The Severe Chronic Neutropenia International Registry (SCNIR) is an important resource for studies on genetic and molecular bases for disorders causing severe chronic neutropenia.

Approximately 60-80% of SCN cases are associated with inherited or spontaneous point mutation in one copy of the gene encoding neutrophil elastase ELA2. This mutation is found in the analysis of congenital neutropenia (CN) families with autosomal dominant inheritance.

Although neutrophil elastase mutations have been found in a subgroup of patients with Kostmann syndrome, as in cyclic neutropenia. Adverse events include the development of acute myeloid leukemia in approximately 7% of the patients within the cohort of patients with Kostmann's syndrome suggesting that congenital neutropenia is a preleukemia syndrome. None of the patients with cyclic of idiopathic neutropenia developed leukemia.[1, 6]

A subset of SCN patients harbor acquired somatic mutations in the CSF3R gene; encoding the granulocyte colony-stimulating factor receptor (G-CSF-R), which has shown a strong predisposition to acute myeloid leukemia[7, 8].These mutations truncate the intracellular domain, producing strong hyper-proliferation, but with defective maturation due to defective internalization and loss binding sites for several negative regulators, thus lead to extended signaling especially of STAT5 (Signal transducer and activator of transcription 5)[9-12]. Other studies reported a further subset of SCN patients, with constitutive mutations in the extracellular domain of the G-CSF-R that collectively act in a dominant-negative manner leading to

hypo-responsiveness to G-CSF.[13, 14]

Studies of the G CSF gene have shown that mutation occur mainly within a critical region of the intracellular part of the receptor.[16, 18]

Using a positional cloning approach and candidate gene evaluation in autosomal recessive severe congenital neutropenic patients (Kostmann disease) were identified a recurrent homozygous germ line mutation in HAX1. HAX1 encodes the mitochondrial protein HAX1, which has been assigned functions in signal transduction and cytoskeletal control. HAX1 is a critical for maintaining the inner mitochondrial membrane potential and protecting against apoptosis in neutrophil development, HAXI deficiency causes autosomal recessive Kostmann disease.[15, 33]

Diagnosis:

Severe Congenital Neutropenia is usually detected in infancy after fever or signs of severe infection develop. Patients have ANCs continuously below 0.2 x 109/L; in many cases, peripheral blood neutrophils are completely absent. With infection, there may be a transient increase in neutrophils, but counts rarely increase to normal levels. Repeated differential blood counts indicate persistent ANCs within a range of 0 to 0.2 x 109/L is required for diagnosis. Blood counts often indicate mild anemia and thrombocytosis. There is usually a two to four fold increase in blood monocytes and an increase in the blood eosinophils count is common. IgG levels are elevated in the majority of patients. The specific immunologic competence after vaccination is normal. Blood chemistry is within the normal, age dependent range for electrolytes, kidney and liver functions. Testing for anti-neutrophil antibodies is helpful to exclude autoimmune neutropenia of infancy.

The bone marrow usually shows maturation arrest of neutrophil precursors at an early stage (Promyelocyte/myelocyte level) with few cells of the neutrophilic series. Promyelocytes often have morphologically atypical nuclei and vacuolization of cytoplasm. The absolute number of promyelocytes is slightly increased. Megakaryocytes are normal in number and morphology. In vitro growth of granulocyte macrophage progenitor cell is defective, with few colonies formed and evidence for "Maturation arrest". Cytogenetic at the time of diagnosis of CN are almost always normal. Bone marrow cytogenetics may change during the course of the disease, with monosomy 7 being the most frequent aberration in about 50% of abnormal cytogenetic results. The interval from the original finding of normal cytogenetics to the appearance of an abnormality is often several years. Abnormal cytogenetics is often associated with morphologic changes of the bone marrow indicating the development of MDS or Leukemia.

Differential Diagnosis:

The differential diagnosis of CN includes a number of congenital and acquired diseases[17]. The most common difficulty is determining whether the patient has cyclic neutropenia, autoimmune neutropenia of infancy, or idiopathic neutropenia. Cyclic neutropenia is diagnosed by serial measurement of blood neutrophils on at least 3 days per week for at least 6 weeks, graphing the

count. Cyclic neutropenia is also a hereditable disease. Family and genetic studies show that it is an autosomal dominant disorder[19]. An important differential diagnostic evaluation is testing for neutrophilic antibodies[20]. Although these infants lack peripheral blood neutrophils, the marrow function is normal; maturation of myeloid precursors to mature neutrophil stage is usually observed. Neutrophil or granulocyte specific antibodies in serum are detectable using immunologic test. Idiopathic neutropenia is a form of acquired neutropenia, it includes patients without evidence of congenital, neoplastic, or immunological causes of neutropenia, the clinical phenotype is similar to autoimmune neutropenia. Patients are categorized as having idiopathic neutropenia unless they are positive for anti neutrophil antibodies.[21, 22]

Therapy:

Severe CN is often recognized when patients present with life-threatening infection during the few months of life and are found to have extremely low circulating neutrophils. Antibiotic therapy for these infections follows the same principles as for other patients: broad-spectrum antibiotics for severe infections until the pathogen is identified; targeted therapy for specific pathogens; administration of antibiotics until the signs and symptoms of infection have cleared. Prophylactic antibiotics may be considered, but are required infrequently since the advent of G-CSF in 1987[23]. Recombinant human granulocyte colony-stimulating factor (rHuG-CSF) requires long-term daily administration to maintain clinical benefit. The ANC should not be the sole indicator of clinical efficacy. Individual adjustment of dosages on the basis of both the patient's clinical course and the ANC. The efficiency of rHuG-CSF has been demonstrated by increasing the number of neutrophils with reduction of infection[23, 24]. In contrast, GM-CSF treatment did not led to an increase in blood neutrophils, but only in blood eosinophils[25]. In 1994, the SCNIR collected data from more than 600 patients with CN, results demonstrated that more than 95% responded to rHuG-CSF treatment with an increase in ANCs to 1.0 x ≥109/L.3 Most CN patients responded to a dose between 3 and 10 mg/kg/d.[16,17]

Generally, rHuG-CSF should be initiated at 5 mg/kg/day, the dose should be escalated to 10 mg/kg/day and then by increments of 10 mg/kg/day at 14-days intervals if the ANC remains below 1.0 x 10^9/L. As soon as the ANC can be maintained at 1.0 to 1.5 x 10^9/L or above, further increase in rHuG-CSF dose can be stopped since the occurrence of bacterial infection is reduced dramatically at this level. The dose of rHuG-CSF can be reduced if the ANC increases to ≥5.0 x 10^9/L to keep the patient at the lowest dose necessary for maintaining sufficient neutrophil count to overcome infections.[23, 29]

Non responders to rHuG-CSF are defined by failure to benefit at dose levels exceeding 120 mg/kg/day. Partial responders have ANCs increased to 0.5 to 1.0 x 10^9/L but still have bacterial infections. In some patients, the dose of rHuG-CSF cannot be increased to these levels because of the large volume and frequency of injections required. Patient was refractory to standard and high dose G-CSF therapy, but responded to a combined corticosteroid/G-CSF treatment, apparently through synergis-

tic activation of STAT5 and stimulate G-CSF-induced cell proliferation.[26, 27]

All responding patients require significantly fewer antibiotics and fewer days of hospitalization. Hematopoietic stem cell transplantation (HSCT) remains the only currently available treatment for patients who are refractory to rHuG-CSF treatment or in those with leukemic transformation.[28]

Adverse events to rHuG-CSF therapy include mild splenomegaly, osteoporosis and malignant transformation into MDS/Leukemia. If and how G-CSF treatment impacts on these adverse events is not fully understood. The hazard of MDS/Leukemia increases significantly overtime.

In the study of Rosenberg, et al.[29], the hazard of MDS/AML increased significantly during the period of observation of SCN on G-CSF, from 2.9% per year after 6 years to 8.0% per year after 12 years. The cumulative incidence of MDS/AML was 21% after 10 years. The risk of MDS/AML increased with the dose of G-CSF. Less responsive patients, defined as those requiring greater than 8 mg/kg/d of G-CSF, had a cumulative incidence of MDS/AML of 40% after 10 years and compared to 11% of more responsive patients. The data were interpreted as indicating that a poor response to G-CSF defines an "at-risk" population and predicts an adverse outcome.[29]

Conversion to MDS/AML in CN patients was associated with one or more cellular genetic abnormalities (Such as monosomy[7] and as mutation or G-CSF-R mutation), which may be useful for identifying subgroups of patients at high risk. Marrow cells from approximately 75% of the severe CN cases that transformed to MDS/AML also show point mutations in the gene for the G-CSF-R; resulting in a truncated C-terminal cytoplasmic region of the receptor that is crucial for maturation signaling[30]. G-CSF therapy can cause cataract and this adverse event is dose related.[31]

CONCLUSION

The use of rHuG-CSF remains first-line treatment for most CN patients, which greatly improved patient's quality of life.

All CN patients, regardless of their treatment or response, are at risk to develop MDS/ or leukemia at an actual evidence of 11.5% and accumulative incidence of 21% after 10 years. Careful monitoring for cytogenetic abnormalities and G-CSF-R mutation is necessary to initiate. Hematopoietic Stem Cell Transplant (HSCT) should be restricted to G-CSF non-respond and leukemic transformation.

ACKNOWLEDGEMENTS

The author would like to extend sincere thanks for Mrs. Susana de Guzman for her assistance in writing this article.

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